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Sentinel Riparian Spiders Predict Mercury Contamination of Riverine Fish

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Abstract: Mercury (Hg) is a widespread and toxic environmental contaminant. It is challenging to determine the level of Hg contamination of food chains and fish within the millions of water bodies in the United States. Mercury contamination can vary 10-fold between ecosystems, even those in the same region. Therefore, aquatic ecosystems need to be individually monitored for Hg contamination to determine which ecosystems are most contaminated and pose the greatest risk to human and wildlife health. One approach to monitoring Hg is to use sentinel species, defined as biological monitors that accumulate a contaminant in their tissues without significant adverse effects. Riparian spiders such as long-jawed orb weavers (Tetragnathidae) have been proposed as sentinels of persistent bioavailable contaminants, like Hg, in aquatic systems. Long-jawed orb weavers feed on emergent aquatic insects and have concentrations of Hg that reflect levels of Hg contamination in the aquatic food web. Previous studies have documented elevated contaminant concentrations in long-jawed orb weavers from shorelines of aquatic ecosystems, suggesting that they could be used as sentinels of chemical contaminants in aquatic ecosystems. We demonstrate for the first time that long-jawed orb weavers can be used as sentinels to identify aquatic systems that contain fish with elevated concentrations of Hg. *Environ Toxicol Chem* 2022;41:1297–1303. © 2022 SETAC

Keywords: Sentinel; Riparian spiders; Mercury; Trophic transfer; Bioaccumulation

INTRODUCTION

Mercury (Hg) is a widespread and toxic environmental contaminant (Driscoll et al., 2013). It is challenging to determine Hg contamination of food chains and fish within the millions of water bodies in the United States (McDonald et al., 2012). Mercury contamination can vary 10-fold between water bodies, even in the same region (Drenner et al., 2011). Therefore, water bodies need to be individually monitored for Hg contamination to determine which are most contaminated and pose the greatest risk to human and wildlife health.

One approach to monitoring Hg is to use sentinel species, defined by Beeby (2001) as biological monitors that accumulate a contaminant in their tissues without significant adverse effects. Fish are commonly utilized as sentinels and have revealed much about contaminants in aquatic systems including the risks posed to human and wildlife health (Chumchal et al., 2022; Wiener et al., 2003). However, fish can be labor-intensive to capture, can have migratory behavior and home ranges that could extend far beyond areas of contamination (see Fry & Chumchal, 2011), and are absent from temporary aquatic systems that periodically dry (Chumchal et al., 2022; Chumchal & Drenner, 2015).

Riparian spiders have been proposed as sentinels of persistent bioavailable contaminants like Hg in aquatic systems because they are widely distributed, seasonally abundant, and easy to capture and have limited home ranges and therefore reflect localized contamination (Chumchal et al., 2022; Gann et al., 2015; Tweedy et al., 2013; Walters et al., 2008). Most studies of riparian spiders as sentinels of Hg contamination have focused on long-jawed orb weavers (Tetragnathidae; Chumchal et al., 2022). Long-jawed orb weavers feed on emergent aquatic insects (Ortega-Rodriguez et al., 2019; Speir et al., 2014), have concentrations of Hg in their tissues that reflect levels of Hg contamination in the aquatic food web (Tweedy et al., 2013), and are an important prey item for songbirds, especially during the breeding season when energy-rich spiders are consumed by both adults and nestlings (Chumchal et al., 2022; Cristol et al., 2008; Walters et al., 2010). Although several studies have documented elevated

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contaminant concentrations in long-jawed orb weavers from the shorelines of previously monitored aquatic ecosystems (Chumchal et al., 2022), no studies have used long-jawed orb weavers as sentinels to identify aquatic ecosystems that contain fish with elevated concentrations of chemical contaminants.

In the present study, we conducted a two-phase study of Hg contamination of long-jawed orb weavers and bluegill (*Lepomis macrochirus*) in the Clear Fork and West Fork of the Trinity River, Fort Worth, Texas, USA. The objective of the first phase in 2016 was to survey Hg concentrations in long-jawed orb weaver spiders from the Clear Fork and West Fork of the Trinity River. We found that long-jawed orb weaver spiders from the Clear Fork had higher concentrations of Hg than spiders from the West Fork, suggesting that the aquatic food webs of the two rivers have different levels of Hg contamination. During the second phase in 2019, we surveyed long-jawed orb weaver spiders along the two rivers and found a pattern of Hg contamination similar to the pattern observed in 2016. To test the hypothesis that Hg contamination of the aquatic food webs differed between the two rivers, we collected bluegill in 2019 from both rivers and found that, like long-jawed orb weavers, bluegill from the Clear Fork had elevated concentrations of Hg relative to the West Fork. Our study is the first to demonstrate that sentinel riparian spiders such as long-jawed orb weavers can be used to identify previously unmonitored aquatic systems that contain fish with elevated concentrations of Hg.

METHODS

Study sites

The Clear Fork and West Fork of the Trinity River in Fort Worth, Texas, USA, are channelized and leveed rivers located downstream from large impoundments. The Clear Fork receives water from Benbrook Lake, whereas the West Fork receives water from Lake Worth and Eagle Mountain Lake, located immediately upstream from Lake Worth (Figure 1). Deliberate impoundment of water in Benbrook Lake, Lake Worth, and Eagle Mountain Lake began in 1952, 1914, and 1934, respectively (Texas Water Development Board [TWDB], 2021a, 2021b, 2021c). We are not aware of any historic or contemporary point sources of Hg into the Clear Fork or West Fork and assume that most of the Hg in the two rivers is from atmospheric deposition of inorganic Hg into the rivers, reservoirs, and their watersheds. The watersheds are located in the Cross Timbers, an ecoregion that receives low levels of wet Hg deposition relative to other ecoregions in north Texas (Drenner et al., 2011). In aquatic systems, inorganic Hg is converted by bacteria to methylmercury (MeHg; Hsu-Kim et al., 2013; Selin, 2009), which is a bioavailable and potent toxin that has adverse effects on vertebrate health (Driscoll et al., 2013; Eagles-Smith et al., 2018). Methylmercury enters the food chain and biomagnifies, reaching high concentrations in top predators such as fish (Lavoie et al., 2013).

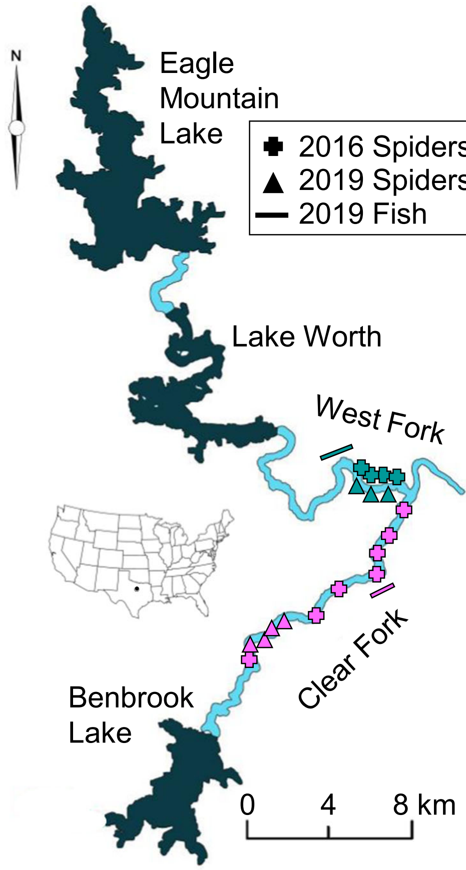

FIGURE 1: Location of sampling sites for spiders collected in 2016 and 2019 and fish collected in 2019 on the Clear Fork and West Fork of the Trinity River, Fort Worth, Texas, USA. Spiders were collected at discrete sampling sites as indicated, while fish were collected from segments of the rivers represented by bars in the figure. The Clear Fork and West Fork sampling sites are downstream from Benbrook Lake and Lake Worth and Eagle Mountain Lake, respectively.

Collection and processing of long-jawed orb weavers

Spiders were collected in two phases, in 2016 and 2019. During Phase 1 of the present study, long-jawed orb weavers were collected from riparian vegetation within 2 m of the shoreline using sweep nets from seven sites on the Clear Fork on June 8, July 8, and July 11, 2016, and four sites on the West Fork on July 8, 2016 (Figure 1). During Phase 2, spiders were collected within 2 m of the shoreline by hand from four sites on the Clear Fork on June 20 and 28 and July 5 and 12, 2019, and three sites on the West Fork on June 13 and 21 and July 2, 2019 (Figure 1). Samples were preserved in 95% denatured ethanol. Ethanol is commonly used to preserve invertebrates prior to Hg analysis and does not affect Hg concentrations (Hannappel et al., 2021). In the laboratory, ethanol-preserved long-jawed orb weavers collected by sweep nets were separated from incidentally captured insects and vegetation. In 2016, 98 and 106 long-jawed orb weavers and, in 2019, 411 and 353 long-jawed orb weavers were collected from the Clear Fork and West Fork, respectively (Supporting Information, Table S1).

We used leg length as a proxy for body size in long-jawed orb weaver spiders because leg length does not change with feeding or reproductive status like other body size measurements (e.g., mass or abdomen size [Danielson-François et al., 2002]) and Hg concentration in long-jawed orb weavers are positively correlated with leg length (Hannappel et al., 2021). In 2016 we measured the length of the tibia of the first leg, but in 2019 (because of a change in our laboratory's standard protocols), we measured the length of the tibia + patella of the first leg (Supporting Information, Figures S1 and S2). In long-jawed orb weavers, the length of the tibia is highly correlated with the length of the tibia + patella (Supporting Information, Figure S1), so we adjusted the leg length data collected in 2016 (tibia only) to be compatible with 2019 leg length data (tibia + patella) using the equation tibia + patella length (mm) = (1.06 × tibia length [mm]) + 0.49. Leg length was measured using ImageJ (Schneider et al., 2012).

Prior to Hg analysis, long-jawed orb weavers were grouped by size into composite samples such that the leg length of the largest spider was no more than 1 mm longer than the smallest spider in the composite. The only exceptions were two samples collected in 2016, which, because of small sample sizes, we combined into composites in which individuals varied by as much as 2.6 mm in leg (tibia + patella) length (12.2–14.2 mm and 11.5–14.1 mm in the Clear Fork and West Fork, respectively). In 2016 spiders were composited by leg length regardless of sampling location, resulting in 10 composite samples from the Clear Fork and West Fork. Spiders collected in 2019 were handled as described above, but because we collected more spiders, we created composite samples based on leg length at each sampling location. After Hg analysis, we averaged composites of similar size class (one to four composite samples per size class on each river) to create 14 and 15 composite samples for the Clear Fork and West Fork, respectively, in 2019.

Collection and processing of bluegill

During Phase 2 of the present study, we collected bluegill to determine if the aquatic food webs of the two rivers differed in their level of Hg contamination. Bluegill are commonly found in water bodies of the south central United States (D. Lee, 1980) and have concentrations of Hg that are intermediate among fish in the region (Chumchal et al., 2011; Fry & Chumchal, 2012). Bluegill were collected using hook and line from approximately 1.25- and 1.75-km segments on the Clear Fork and West Fork, respectively (Figure 1). Fifty-five and 75 bluegill were collected on July 1, 3, 24, and 25 and July 21, 22, and 23, 2019, from the Clear Fork and West Fork, respectively; euthanized in a solution of tricaine methanesulfonate anesthetic; and frozen. In the laboratory, bluegill were thawed and measured for body size. We used total length as a proxy for body size in bluegill because total length is a standard measurement of body size in fish and Hg concentrations in bluegill are positively correlated with total length (see Chumchal & Hambright, 2009). After Hg analysis, we created composite samples based on bluegill size such that the total length of the largest fish was no more than 1 cm longer than the smallest fish in the composite. This resulted in seven and eight composite bluegill averages for the Clear Fork and West Fork, respectively.

Hg analyses

We analyzed total Hg in spiders and fish as a proxy for MeHg concentration. A review of six studies found that in tetragnathid spiders 70.8 ± 1.79% (average ± SE) of total Hg is MeHg (Hannappel et al., 2021). Most (>95%) of the total Hg in fish is MeHg (Bloom, 1992). Composite samples of whole long-jawed orb weaver spiders and epaxial muscle from individual bluegill were dried in a 60° oven for 48 h and then analyzed for total Hg using a Milestone DMA-80 Direct Hg Analyzer, which uses thermal decomposition, gold amalgamation, and atomic-absorption spectroscopy (US Environmental Protection Agency, 1998). Quality assurance included reference standards (National Research Council of Canada Institute for National Measurement Standards) and duplicate samples. Reference standards (DORM-4 in 2016 and 2019 and PACS-2 in 2019) were analyzed every 10 samples; PACS-2 is a high total Hg standard and was only analyzed in 2019 along with fish samples which contained relatively high concentrations of Hg. The average recovery percentage for DORM-4 in 2016 and 2019 was 95.8 ± 0.69% (mean ± SE, $n = 4$) and 95.6 ± 0.72% (mean ± SE, $n = 36$), respectively. The average recovery percentage for PACS-2 in 2019 was 112 ± 3.4% (mean ± SE, $n = 3$). Duplicate samples were analyzed every 20 samples. The average relative percentage difference between duplicate spider samples analyzed in 2016 and 2019 was 2.1 ± 0.01% (mean ± SE, $n = 2$) and 2.3 ± 0.55% (mean ± SE, $n = 11$), respectively. The average relative percentage difference between duplicate fish samples analyzed in 2019 was 11.7 ± 3.17% (mean ± SE, $n = 12$).

Statistics

We used analysis of covariance models to determine the effect of river (categorical variable) and body size (covariate) on total Hg concentrations in long-jawed orb weavers and bluegill. Body size in long-jawed orb weavers and bluegill was measured as leg (tibia + patella) length and total length, respectively. We first tested for an interaction effect between river and body size. Significant river × body size interactions were identified in all models (indicating heterogeneous slopes), so we also assessed (1) the effect of body size on total Hg concentrations within each river using simple linear regression, and (2) the effect of river on total Hg concentration using an F test based on the linearly independent pairwise comparisons among estimated marginal means. We used analysis of variance (ANOVA) followed by Fisher's least significant difference test to determine if maximum water surface elevation changes differed between the three reservoirs upstream of the two rivers. Statistical significance was determined at $p < 0.05$, and statistical tests were performed using SPSS.

RESULTS

In 2016 and 2019, we detected a significant leg length × river interaction effect on total Hg concentrations in spiders (Figure 2A and B and Table 1; $p < 0.001$), indicating that the effects of leg length and river on total Hg concentrations were not independent. Total Hg concentrations in spiders were positively correlated with leg length in the Clear Fork in both 2016 and 2019 (2016, $R^2 = 0.60$, $F_{1,8} = 11.9$, $p = 0.009$; 2019, $R^2 = 0.79$, $F_{1,12} = 49.5$, $p < 0.001$), but total Hg concentrations in spiders were not related to leg length and were inversely related to leg length in the West Fork in 2016 and 2019, respectively (2016, $R^2 = 0.10$, $F_{1,8} = 0.85$, $p = 0.38$; 2019, $R^2 = 0.31$, $F_{1,13} = 5.75$, $p = 0.032$; Figure 2A and B). Estimated marginal mean total Hg concentrations of spiders from the Clear Fork were 1.6 and 2.4 times higher than those from the West Fork in 2016 and 2019, respectively (2016, $F_{1,16} = 46.9$, $p < 0.001$, total Hg differences evaluated at 6.9 mm leg length; 2019, $F_{1,25} = 328$, $p < 0.001$, total Hg differences evaluated at 9.2 mm leg length; Figure 2A and B). Based on these results, we hypothesized that the food web of the Clear Fork had higher concentrations of Hg than the food web of the West Fork.

To test the above hypothesis, we assessed total Hg concentrations in bluegill from the two rivers. We detected a significant total length × river interaction effect on total Hg concentrations in bluegill (Figure 2C and Table 1; $p < 0.001$), indicating that the effects of bluegill total length and river on total Hg concentrations were not independent. Total Hg concentrations in fish were positively correlated with total Hg in the Clear Fork ($R^2 = 0.77$, $F_{1,5} = 16.5$, $p = 0.01$) but not the West Fork ($R^2 = 0.48$, $F_{1,6} = 5.44$, $p = 0.06$; Figure 2C). Like with spiders, estimated marginal mean total Hg concentrations of bluegill from the Clear Fork were 2.6 times higher than those from the West Fork ($F_{1,11} = 92.0$, $p < 0.001$, total Hg differences evaluated at 13.7 cm total length; Figure 2C).

DISCUSSION

Since Walters et al. (2008) proposed long-jawed orb weavers as sentinels of chemical contaminants in aquatic systems, 27 studies have demonstrated that long-jawed orb weavers in riparian habitats accumulate contaminants in their tissues and thus have potential as sentinels (Chumchal et al., 2022). Most of these studies have documented elevated contaminant concentrations in spider tissues from the shorelines of previously monitored aquatic ecosystems (Chumchal et al., 2022). We are aware of only one study that used riparian spiders (bridge spiders, *Larinioides sclopetarius*) as sentinels of Hg in previously unmonitored systems (Pennuto & Smith, 2015).

In the present study, we discovered that long-jawed orb weavers along the Clear Fork and West Fork had different levels of Hg contamination, leading us to conduct field studies to determine if similar differences in Hg contamination would be observed in fish. We found that long-jawed orb weavers and bluegill in the Clear Fork had Hg concentrations that were approximately 2.5 times greater than in the West Fork, demonstrating that long-jawed orb weavers can be used to predict Hg contamination of fish. These results suggest that spiders can be utilized as cost-effective sentinels to identify aquatic systems that are highly contaminated with Hg and are therefore candidates for additional monitoring of aquatic biota, including fish.

We considered three alternative hypotheses to explain why the Clear Fork had higher Hg contamination than the West Fork.

Land cover

Landscape factors affect the sensitivity of the environment to atmospheric deposition of inorganic Hg (Driscoll et al., 2007; Evers et al., 2007; Wiener et al., 2003). Mercury-sensitive

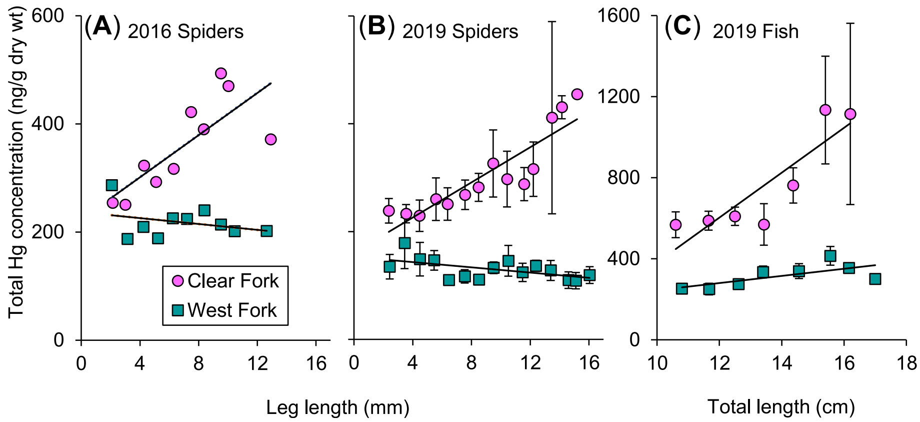

FIGURE 2: Relationship between leg length (tibia + patella) and total Hg concentrations in long-jawed orb weavers collected in 2016 (**A**) and 2019 (**B**) and total length and total Hg concentrations in bluegill collected in 2019 (**C**) from the Clear Fork and West Fork of the Trinity River. Error bars equal standard error.

TABLE 1: Model parameters from analysis of covariance models assessing two-way interactions and main effects of river and body size (leg length [tibia + patella] for long-jawed orb weavers and total length for bluegill) on total Hg concentrations

		Covariate × river			River			Covariate		
Dependent variable	Covariate	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Total Hg in spiders (2016)	Leg length	1,16	13	0.003	1,16	0.1	0.8	1,16	7.4	0.15
Total Hg in spiders (2019)	Leg length	1,25	60	<0.001	1,25	0.1	0.76	1,25	33	<0.001
Total Hg in fish (2019)	Total length	1,11	13	0.004	1,11	5.1	0.05	1,11	13	0.004

landscapes are those in which relatively small inputs of inorganic Hg can cause significant contamination of fish with MeHg (Driscoll et al., 2007; Wiener et al., 2003). Some land-cover types that affect the transport and methylation of Hg, such as forests and wetlands, respectively, promote Hg contamination of aquatic food webs, while other land-cover types associated with agriculture may decrease Hg contamination of aquatic biota (Driscoll et al., 2007). Using the 2001 National Land Cover Dataset, T. Lee et al. (2015) found that range land was the dominant land cover of the watersheds of Benbrook Lake and Eagle Mountain Lake. Range land covered 64.5% and 58.5% of the watersheds of Benbrook Lake and Eagle Mountain Lake, respectively, followed by <20% coverage by forests in both watersheds. Because atmospheric Hg deposition and land cover of the watersheds are similar, we conclude that it is unlikely that differences in Hg deposition and land cover contributed to the differences in Hg contamination of the two rivers.

Nutrients and trophic state

Nutrients such as phosphorus and trophic state have been hypothesized to affect Hg concentrations in biota and fish (Chételat et al., 2020; Driscoll et al., 2013). Both experimental and field studies suggest that nutrient enrichment leading to algal blooms diminishes Hg bioaccumulation in phytoplankton, a process known as *biodilution* (Driscoll et al., 2013). Concentrations of Hg in zooplankton also decrease with increasing zooplankton densities associated with elevated nutrient concentrations, which in turn are correlated with lower Hg concentrations in fish (Driscoll et al., 2013). Growth dilution in fish occurs when productivity and food availability are high and may also result in lower Hg concentrations in fish (Driscoll et al., 2013). We assume that the reservoirs supplying water to the rivers are primarily responsible for the nutrient status of the two rivers. All three reservoirs are eutrophic and had concentrations of total phosphorus of 0.06 mg/L (Texas Commission on Environmental Quality, 2019). Therefore, we conclude that it is unlikely that differences in phosphorus and trophic state of the reservoirs contributed to the differences in Hg contamination between the Clear Fork and West Fork.

Operation of reservoir dams

The difference in Hg contamination of the two rivers may be due in part to the design and operation of reservoir dams and associated water-level fluctuations in the reservoirs immediately above our sampling sites (Figure 1). Between-year fluctuations in maximum water storage of reservoirs are positively correlated with total Hg concentrations in fish (Willacker et al., 2016). Eckley et al. (2015) found that reservoir water-level fluctuations can affect sediment redox conditions and enhance MeHg production. This process can result in a continued increase of MeHg concentrations in older reservoirs after the initial impact of landscape flooding has subsided (Eckley et al., 2015).

Benbrook Lake is used for flood control of the lower Clear Fork (TWDB, 2021b), which increases water-level fluctuations in the reservoir, while Lake Worth is not designed to store floodwater flowing down the West Fork (TWDB, 2021a). Lake Worth is normally a relatively constant-level riverine lake and is operated as a pass-through reservoir (TWDB, 2021a). Water-level fluctuations in Lake Worth are further reduced by water input from an upstream reservoir, Eagle Mountain Lake (Figure 1). Water is discharged from Eagle Mountain Lake downstream into Lake Worth to maintain a minimum water level in Lake Worth that will allow water output to water-treatment facilities in Fort Worth (M. Ernst, Tarrant Regional Water District, Fort Worth, Texas, USA, [personal communication, October 13, 2021]).

To examine water-level fluctuations in the three reservoirs, we used US Geological Survey (USGS) data (USGS, 2021) to compute between-year changes in water surface elevations in Benbrook Lake (USGS site no. 08046500 Benbrook Lk), Lake Worth (USGS site no. 08045400 Lk Worth), and Eagle Mountain Lake (USGS site no. 08045000 Eagle Mtn Res) for the years 2012–2019. Mean between-year fluctuations in water surface elevation differed significantly between reservoirs (ANOVA $F_{2,18} = 4.62$, $p = 0.02$) and were greater in Benbrook Lake than either Lake Worth or Eagle Mountain Lake (Fisher's least significant difference $p < 0.03$; Figure 3).

We hypothesize that fluctuations in water surface elevation of Benbrook Lake led to increased MeHg production and discharge of MeHg into the Clear Fork, increasing both the absolute amount of MeHg and the MeHg to Hg(II) ratio in the Clear Fork relative to that in West Fork. Compared to Hg (II), MeHg has a higher assimilation efficiency and a slower elimination rate, which can result in greater bioaccumulation (Wang & Wong, 2003). Higher concentrations of MeHg or a higher MeHg to total Hg ratio in prey of spiders and fish in the Clear Fork than prey in the West Fork would result in positive relationships between size and Hg concentration in spiders and fish from the Clear Fork but not the West Fork.

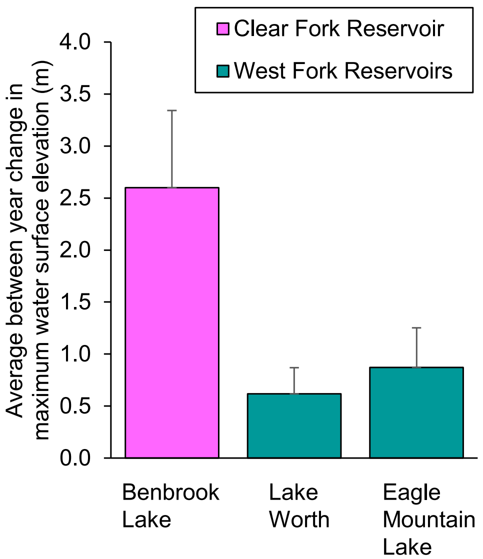

FIGURE 3: Average (±standard error) between-year changes in maximum water surface elevation from 2012 to 2019 for Benbrook Lake, Lake Worth, and Eagle Mountain Lake. Benbrook Lake is located upstream of the Clear Fork sampling sites, and Lake Worth and Eagle Mountain Lake are located upstream of the West Fork sampling sites (Figure 1).

CONCLUSION

In conclusion, a number of studies have documented elevated contaminant concentrations in spider tissues from the shorelines of previously monitored aquatic ecosystems (Chumchal et al., 2022), but only one study has used spiders to survey unmonitored aquatic water bodies for Hg contamination (Pennuto & Smith, 2015). In the present study, we used riparian long-jawed orb weavers to reveal different levels of Hg contamination of the Clear Fork and West Fork of the Trinity River. Bluegill samples confirmed that the Clear Fork had higher Hg contamination levels than the West Fork. Collecting a range of sizes of both spiders and fish was essential to identifying differences in Hg contamination of the two rivers because it allowed for the comparison of Hg contamination in similar-sized organisms. Our's is the first study to demonstrate that riparian spiders can be used as sentinels to predict Hg contamination of fish from a previously unmonitored river.

Supporting Information—The Supporting Information is availableon the Wiley Online Library at https://doi.org/10.1002/etc.5307.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (m.m.chumchal@tcu.edu).

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